

is SEQ ID NO:20) or a mutant of a naturally occurring homologue of P450<sub>cam</sub>, typically of P450<sub>BM-3</sub> from *Bacillus megaterium* (such as a mutant of the sequence shown in table 8; SEQ ID NO:2), P450<sub>terp</sub> from *Pseudomonas sp.*, and P450<sub>eryF</sub> from *Saccharopolyspora erythraea*, and also P450 105 D1 (CYP105) from *Streptomyces griseus* strains. Note that the amino acid numbering shown in table 8 for P450<sub>BM-3</sub> (SEQ ID NO:21) does not correspond to the numbering used in the description to denote mutations in this enzyme. The sequence shown in table 8 contains an additional amino acid at the N terminal. This is normally cleaved in vivo. Therefore each amino acid number shown in the table is always one more than the number used in the conventional numbering (as used in the description).

**Page 18, paragraph bridging page 17 and 18:**

The second type of cell expresses:

- (a) (i) P450<sub>cam</sub> or a fragment thereof; or
  - (ii) a naturally occurring homologue of P450<sub>cam</sub> or a fragment thereof; or
  - (iii) a mutant of P450<sub>cam</sub>; or
  - (iii) a polypeptide which has at least 70% amino acid homology with (i) or (ii) and optionally has any of the combination of mutations discussed herein; and
  - (b) an electron transfer reductase; and
  - (c) an electron transfer redoxin:
- excluding an *E.Coli* DH5\* cell in which the only mutants of P450<sub>cam</sub> which are expressed are amongst the following:

H<sub>2</sub>N-P450<sub>cam</sub>-TDGTSST (SEQ ID NO:3)-putidaredoxin  
reductase-TDGASSS (SEQ ID NO:4)-putidaredoxin-COOH,

H<sub>2</sub>N-P450<sub>cam</sub>-TDGTRPGPGPGPGPSST (SEQ ID NO:5)-putidaredoxin  
reductase-TDGASSS-putidaredoxin-COOH,

H<sub>2</sub>N-P450<sub>cam</sub>-TDGTRPGPGPGPGPGPSST (SEQ ID  
NO:6)-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H<sub>2</sub>N-putidaredoxin reductases-TDGASSS-putidaredoxin-PLEL (SEQ ID  
NO:7)-P450<sub>cam</sub>-COOH.

**Page 19, third full paragraph:**

Alternatively a linker may be present between the components. The linker generally comprises amino acids that do not have bulky side chains and therefore do not obstruct the folding of the protein subunits. Preferably the amino acids in the linker are uncharged. Preferred amino acids in the linker are glycine, serine, alanine or threonine. In one embodiment the linker comprises the sequence N-Thr-Asp-Gly-Gly-Ser-Ser-Ser-C (SEQ ID NO:8). The linker is typically from at least 5 amino acids long, such as at least 10, 30 or 50 or more amino acids long.

**Page 29, first paragraph under subheading "1.a":**

For the *camA* gene the primer below (SEQ ID NO:9) was used at the 5' end of the gene to introduce the *Eco* RI cloning site and to change the first codon from GTG to the strong start codon ATG.

5'- GAG ATT AAG AAT TCA TAA ACA CAT GGG AGT GCG TGC CAT ATG AAC GCA  
AAC

*Eco* RI          RBS          \*\**camA*

**Page 29, second paragraph under subheading "1.a":**

At the 3' end of *camA* the primer was designed such that 15 bases are complementary to nucleotide sequence of the last five amino acid residues of *camA*. The stop codon immediately after the GCC codon for the last amino acid was removed, and then part of a seven amino acid linker (Thr Asp Gly Gly Ser Ser Ser; SEQ ID NO:8) which contained a *Bam* HI cloning site (GGATCC = Gly Ser; SEQ ID NO:23) was introduced. The coding sequence was thus (nucleic acid sequence is SEQ ID NO:10, amino acid sequence is SEQ ID NO:22):

5'- GAA CTG AGT AGT GCC ACT GAC GGA GGA TCC TCA TCG-3'

*camA*          \* Thr Asp Gly Gly Ser

\**Bam* HI\*

**Page 29, third paragraph under subheading "1.a":**

The primer sequence shown below (SEQ ID NO:11) is the reverse complement used for PCR:

5'- CGA TGA **GGA TCC** TCC GTC AGT GGC ACT ACT CAG TTC-3'

**Page 30, first paragraph under subheading "1.b" and bridging page 29 to 30:**

For the *camB* gene the primer at the 5' end (SEQ ID NO:12) incorporated the second half of the peptide linker between the reductase and redoxin proteins, and the restriction site *Bam* HI for joining the two amplified genes together.

5'- TCA TCG **GGA TCC** TCA TCG **ATG** TCT AAA GTA GTG TAT-3'

Gly Ser Ser Ser \*\* *camB*

\**Bam* HI\*      Start

**Page 30, second paragraph, first full paragraph of page 30:**

At the 3' end of *camB* the primer incorporates 12 nucleotides complementary to the end of *camB* followed by the stop codon TAA, a 6 nucleotide spacer before the GGAG ribosome binding site. *Xba* I and *Hind* III sites were then added to allow cloning of the *camC* gene when required. The sequence of the coding strand (SEQ ID NO:13) was therefore:

5'- CCC GAT AGG CAA TGG **TAA** TCA TCG **GGAG** TCT **AGA** GCA TCG **AAG CTT** TCA TCG-3'

*CamB*    \*\*stop      RBS *Xba* I      *Hind* III

The primer shown below is the reverse complement used for PCR (SEQ ID NO:14):

5'-CGA TGA AAG CTT CGA TGC TCT AGA CTCC CGA TGA TTA CCA TTG  
CCT ATC GGG -3'.

**Page 32, first full paragraph under subheading "2.b":**

We used the internal and unique restriction site *Mlu* I (recognition sequence ACGCGT) within the *camB* gene as the starting point so that the PCR product has a different size from the PCR template fragment. The primers were as follows:

5'- TCA TCG **ACG CGT** CGC GAA CTG CTG-3'

where the *Mlu* I site is in bold (SEQ ID NO:15).

**Page 32, second full paragraph under subheading "2.b":**

The desired coding sequence at the 3' end of the *camB* gene was SEQ ID NO:16:

5'- CCC GAT AGG CAA TGG **TAA** GTA GGT GAA TAT CTA ATC CCC ATC

*camB*      \*\*stop

TAT GCG CGA GTG **GAG TCT** AGA GTT CGA-3'

RBS    *Xba* I

**Page 34 and 35, paragraph bridging pages 34 and 35, second paragraph under heading "3.":**

The primers shown below maintain the *Hind III* cloning site AAGCTT:

5'-TCA TCG **AAG CTT** GGC TGT TTT-3' (SEQ ID NO:17)

*Hind* III \*\* vector

At the other end the coding sequence desired was SEQ ID NO:18:

5'-ACA ATT TCA CAC **AGGA TCT AGA C CAT ATG** TCA TCG **AAG CTT** TCA  
TCG-3'

Vector \*\*RBS *Xba* I    *Nde* I        *Hind* III

This sequence maintained the *Nde* I and *Hind* III sites but the new *Xba* I site was introduced upstream of the *Nde* I site. The PCR primer used was the reverse complement of the desired sequence (SEQ ID NO:19):

5'-CGA TGA AAG CTT CGA TGA CAT ATG GTC T AGA TCCT GTG TGA AAT  
TGT-3'.

Respectfully submitted,



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